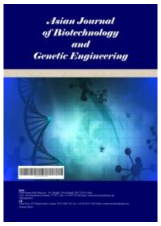

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Bioassay Toxicity Assessment of Remazol Brilliant Blue and Aniline Purple Textile Dyes Contaminated Water Using *Zea mays* and *Sorghum bicolor*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The bioassay evaluation of the toxic textile dyes contaminated water has become highly essential due to indiscriminate discharge of wastewater from the local textile dyes factories in Nigeria and also around the world. Textile dyes wastewater has a reasonable amount of organic and inorganic substances, in many cases high load of heavy metals. In this study, the Remazol brilliant blue (RBB) and Aniline purple (AP) textile dyes contaminated water recorded high biochemical oxygen demand (BOD) and chemical oxygen demand (COD) values above the standard requirement of wastewater discharge. Germination percentage of *Zea mays* and *Sorghum bicolor* seeds decreases with increasing concentration of Remazol brilliant blue and Aniline purple textile dyes water. *Sorghum bicolor* seeds have shown to be more sensitive to the different types and various concentrations of textile dyes compared to *Zea mays*. About 90 and 95% of *Sorghum bicolor* seeds did not germinate in the different types of textile dyes at various concentrations. Lack of further development of germinated seeds to a distinct root and shoot was also observed in *Sorghum bicolor* compared with the *Zea mays* germinated seeds.

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Keywords: Zea mays; root length; Remazol brilliant blue; Aniline purple; Sorghum bicolor.

1. INTRODUCTION

Our immediate environment has been contaminated with different pollutants due to the activities of humans, for example, indiscriminate wastewater disposal from local dyes and textile factories [1,2]. Textile industries and local dyes factories in Nigeria use a lot of water at various processing stages, from scouring, bleaching, mercerizing, dyeing, printing and final finishing [3]. Colour has contributed to the contamination of textile dyes effluent because of the presence of various pigments as shown in Fig. 1. Highly coloured textile dyes wastewaters have the potential to prevent the penetration of solar radiation and oxygen, which is necessary for the survival of various aquatic lives [4]. The removal of colour from textile dyes wastewaters is equally important as remediating the organic substances that could be present because it can influence the fraction of the biochemical oxygen demand (BOD) in the wastewater [5]. Effluents released from these operations usually contain a significant amount of complex pollutants of concern as these are usually discharged to the environment half treated or untreated [5]. The chemical reagents used in dyes and textile industries are made up of inorganic and organic chemicals [6]. Textile dyes and their related allied chemicals have been found to contain higher amounts of heavy metals mainly chromium, copper, lead and cadmium, these types of heavy metals are the primary constituents of colour pigments in textile dyes [7]. In many cases, the presence of heavy metals in waste contaminated water is considered a carcinogenic and mutagenic substance that may likely end up in the food chain [4]. Plant and plant materials have shown potential to be used as good bioindicators of environmental contaminations including textile dyes wastewater due to their differential sensitivity response that could be either susceptible or tolerant [8]. Plant growth parameters such as germination percentage, shoot and root elongation have been used as criteria to assess plant response to specific pollutants.

It was, therefore, essential to have used *Sorghum bicolor* and *Zea mays* seeds to conduct a bioassay analysis using Remazol brilliant blue (RBB) and Aniline purple (AP) textile dyes contaminated water at various concentrations. The objectives were (1) to evaluate the seed germination percentage of *Sorghum bicolor* and *Zea mays* to various concentrations of Remazol Brilliant Blue (RBB) and Aniline purple (AP) textile dyes contaminated water. (2) to observe early seedling shoot and root elongation.

2. MATERIALS AND METHODS

2.1 Phytotoxicity Bioassay

2.1.1 Plant materials and textile dyes

Seed of *Sorghum bicolor* and *Zea mays* was purchased from the Agroseed vendor in Gombe, Gombe State. Healthy uniform size seeds were selected and used for bioassay toxicity evaluation in the Research Lab of Biological Sciences Department, Federal University of Kashere, Gombe, Nigeria. Remazol brilliant blue (RBB) and Aniline purple (AP) textile dyes were purchased from Sabon Gari Market, Kano, Kano State Nigeria.

2.1.2 Biochemical oxygen demand (BOD_5) and chemical oxygen demand (COD) analysis

Biochemical oxygen demand (BOD_5) and chemical oxygen demand (COD) was conducted according to the standard procedure of Association Official Analytical Chemists [9] at the Biochemistry research Laboratory, Gombe State University, Gombe State, Nigeria.

2.1.3 Treatment of seed with water contaminated Remazol Brilliant Blue (RBB) and Aniline purple (AP) textile dyes

Seeds of *Sorghum bicolor* and *Zea mays* were sown in 25 mL of a test solution (0, 1.5, 2.0 and 4% Remazol brilliant blue (RBB) and Aniline purple (AP) textile dyes, prepared in deionised water, w/v) in a glass Petri dish (120 mm diameter). There were three replicates (3 Petri dishes each with 20 seeds) for each of the test solutions of Remazol brilliant blue (RBB) and Aniline purple (AP) textile dyes. All the replicates were arranged in a completely random way on the bench in a growth room at 25 □. Germination (radicle emergence from seed) rates were scored daily up to 7 days from sowing.

2.1.4 Measurement of root length, and shoot height

Root length and shoot height of the seedlings were measured using a ruler after 7 days from sowing seeds from Remazol brilliant blue (RBB) and Aniline purple (AP) textile dyes treatments, respectively.

2.1.5 Statistical analyses

The experiments were designed and conducted using a completely randomised block design (CRBD) with three replications in each treatment. IBM SPSS (version 24) software was used to analyse the data using the analysis of variance (ANOVA) for a randomised block design. The mean difference between the independent replicate at the 5% level of significance was calculated and tested using the *post hoc* test of unprotected least significant difference (LSD). The graph was plotted using the GraphPad Prism software Version 7.0.

3. RESULTS AND DISCUSSION

The biochemical oxygen demand (BOD_5) and chemical oxygen demand (COD) analysis of the experimental test solutions Remazol brilliant blue (RBB) and Aniline purple (AP) textile dyes showed reasonably higher values indicative of extreme pollution load (Table 1). In Nigeria, wastewater discharge quality standards have been set to improve the public health and quality of the environment. Wastewater discharge standard regulations from the National Environmental Standards and Regulations Enforcement Agency (NESREA) have set the standard required values for the BOD_5 and COD effluent discharge limits in Nigeria as 30-50 and 60-90, respectively [10]. In this study, the BOD_5 and COD values found in Table 1 suggest the high content of organic pollutants that may have been present at the various concentrations of all the treatments used.

The seed germination percentage in *Zea mays* and *Sorghum bicolor* water contaminated with Remazol Brilliant Blue (RBB) and Aniline purple (AP) textile dyes at the various concentrations were significantly lower than control (deionised water) (Figs. 2, 3 & 4). For example, the germination percentage of *Zea mays* seeds in 4% RBB treatment was found to be decreased by about 60% when compared with the control (deionised water) (Figs. 2 & 3).

Fig. 1. Waste textile dyes and contaminated land behind Abubakar Tafawa Balewa Stadium, Bauchi, Bauchi State, Nigeria

Table 1. Biochemical oxygen demand (BOD) and chemical oxygen demand (COD) of Remazol brilliant blue (RBB) and Aniline purple (AP) contaminated textile dye water at various concentrations in weight per volume (w/v)

Treatment Remazol brilliant blue (RBB) dye % (w/v)	COD (g/L)	BOD_5 (g/L)	Treatment Aniline purple (AP) dyes % (w/v)	COD (g/L)	BOD_5 (g/L)
1.5	28.11	15.48	1.5	32.36	18.67
2.0	36.14	19.26	2.0	41.83	21.31
4.0	69.29	33.38	4.0	75.68	34.80

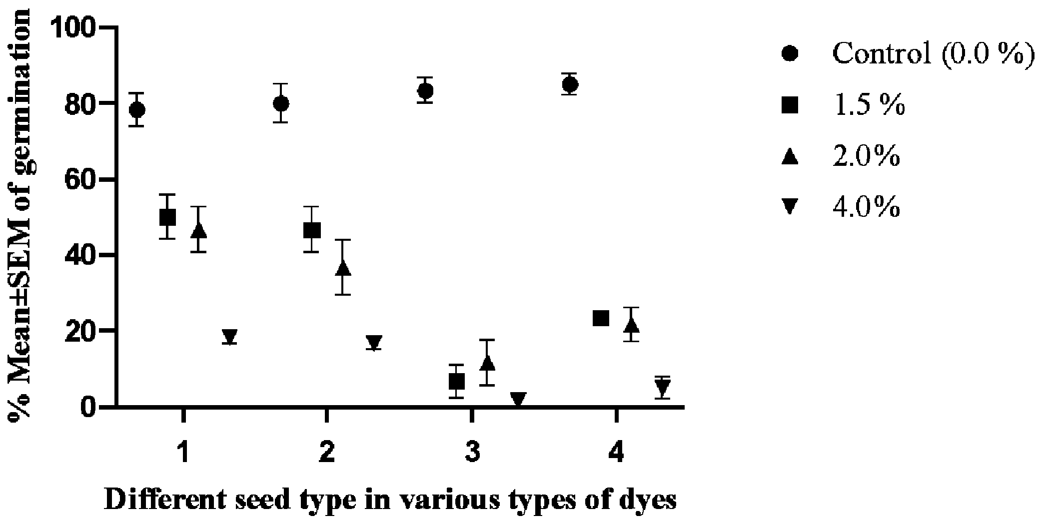

Fig. 2. Mean % germination of *Zea mays* and *Sorghum bicolor* seeds in 0 (control), 1.5, 2.0, and 4.0 (w/v) Remazol brilliant blue (RBB) and Aniline purple (AP) textile dyes contaminated water at one week. Note: 1= *Zea mays* seeds in Remazol brilliant blue textile dye; 2= *Zea mays* seeds in Aniline purple textile dye; 3= *Sorghum bicolor* seeds in Remazol brilliant blue textile dye; 4= *Sorghum bicolor* seeds in Aniline purple textile dye

A B C D

E F G H

Fig. 3. Germination of *Zea mays* and *Sorghum bicolor* seeds in 0 (control; A &E), (1.5; B & F), (2.0; C & G) and (4.0; D & H) (w/v) Remazol brilliant blue (RBB) textile dyes contaminated water at one week. Note: A-D is *Zea mays* and E-H is *Sorghum bicolor* seeds at different concentrations

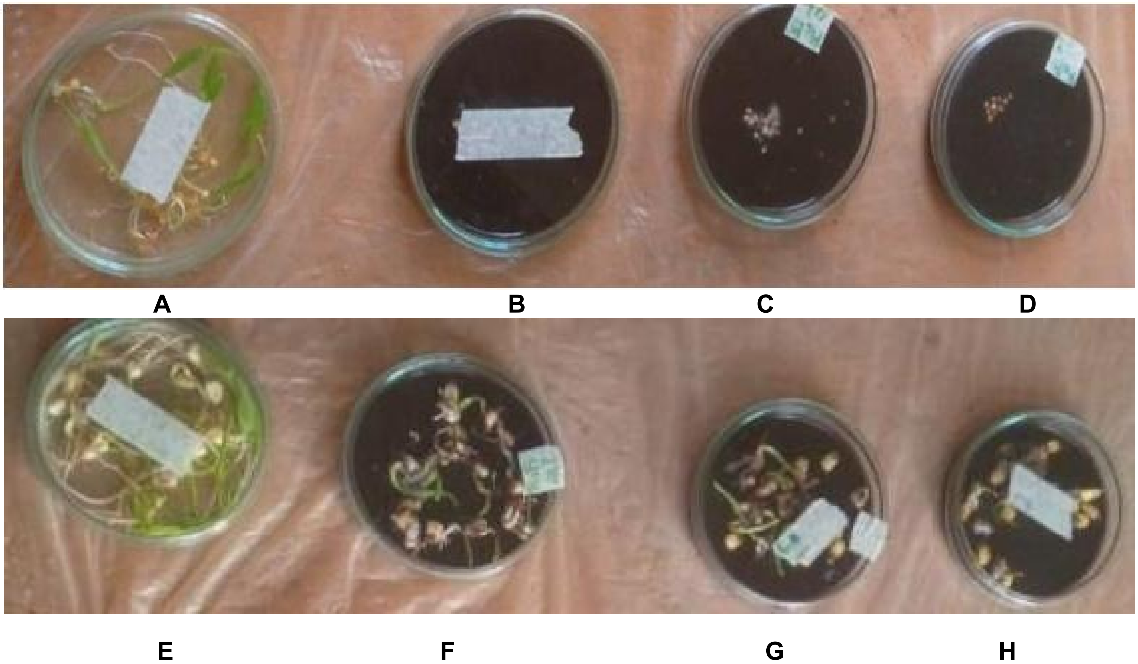

Fig. 4. Germination of *Zea mays* and *Sorghum bicolor* seeds in 0 (control; A &E), (1.5; B & F), (2.0; C & G) and (4.0; D & H) (w/v) Aniline purple (AP) textile dyes textile dyes contaminated water at one week. Note: A-D is *Zea mays* and E-H is *Sorghum bicolor* seeds at different concentrations

Table 2. Shoot height and root length of one week old seedling of *Zea mays* at different concentrations of Remazol brilliant blue (RBB) textile dye contaminated water

Treatment % (w/v)	**0.0**	**1.5**	**2.0**	**4.0**
Root Length (cm)	11.71 ± 1.42 (b)	1.17 ± 0.14 (a)	1.19 ± 0.27 (a)	0.76 ± 0.11 (a)
Shoot Height (cm)	13.06 ± 0.29 (c)	4.17 ± 0.14 (b)	2.87 ± 0.58 (a)	3.93 ± 0.13 (ab)

Values are expressed as Mean ±SEM; Values with different letters across the rows differ significantly at $P<0.05$

Zayneb et al. [11] reported about 50% decreased germination in maize and sorghum seeds treated with untreated wastewater. In another study, untreated wastewater has significantly reduced the germination percentage of alfalfa, fescue and sorghum when compared to control (treated wastewater) [8]. Similarly, the relative reduction in seed germination percentage of clover, wheat, tomato lettuce were also observed [12]. For, example, above 60% of alfalfa and fescue germinated after 3 three days and 50% for *Sorghum bicolor* after 5 days [8].

Here in this study, the percentage germination of *Sorghum bicolor* seed in all the RBB treated water recorded very low with less than 5% germination in 1.5% RBB contaminated water (Fig. 2). In 4% RBB contaminated water no germination was recorded for *Sorghum bicolor* seed but when compared with 4% AP treated water only 2% germination was recorded (Figs. 2 & 4). Previous study showed that the speed of germination of *Sorghum bicolor* seed was strongly reduced but almost unaffected in maize seed [11]. In this study, the results obtained may have suggested the presence of higher concentrations of anionic surfactant that induced a decrease in seed germination percentage of *Sorghum bicolor* [13]. In 1.5% AP contaminated water, 40% of the seed of *Zea mays* germinated (Fig. 4). Generally, in this study, the higher values of BOD and COD in the different textile dyes contaminated water suggested the presence of toxic organic and inorganic substances that has an inhibitory effect on seed germination of *Zea mays* and *Sorghum bicolor*. The colour intensity of effluent is related to the metallic complexity of the treatments [14,15,16].

Table 3. Shoot height and root length of one week old seedling of *Zea mays* at different concentrations of Aniline purple textile dye contaminated water

Treatment % (w/v)	0.0	1.5	2.0	4.0
Root Length (cm)	10.03 ± 1.47(a)	0.00±0.00(c)	0.00±0.00(c)	0.00±0.00(c)
Shoot Height (cm)	13.57 ± 0.14(b)	0.00±0.00(d)	0.00±0.00(d)	0.00±0.00(d)

Values are expressed as Mean ±SEM; Values with different letters across the rows differ significantly at P< 0.05

Table 4. Shoot height and root length of one week old seedling of *Sorghum bicolor* at different concentrations of Aniline purple textile dye contaminated water

Treatment % (w/v)	0.0	1.5	2.0	4.0
Root length (cm)	11.71 ± 1.42(a)	1.66 ± 0.22(b)	1.34 ± 0.15(b)	0.47 ± 0.33(b)
Shoot height (cm)	13.06 ± 0.29(c)	4.81 ± 0.36(a)	4.07 ± 0.22(a)	2.75 ± 1.10(a)

Values are expressed as Mean ±SEM; Values with different letters across the rows differ significantly at P< 0.05

Table 5. Shoot height and root length of one week old seedling of *Sorghum bicolor* at different concentrations of Remazol brilliant blue (RBB) textile dye contaminated water

Treatment % (w/v)	0.0	1.5	2.0	4.0
Root Length (cm)	10.03 ± 1.47(a)	0.00±0.00(b)	0.00±0.00(b)	0.00±0.00(b)
Shoot Height (cm)	13.57 ± 0.14(c)	0.00±0.00(d)	0.00±0.00(d)	0.00±0.00(d)

Values are expressed as Mean ±SEM; Values with different letters across the rows differ significantly at P < 0.05)

Textile dyes vary in chemical composition, however, the shared common characteristics such as the presence of metal complexes predominantly traces of heavy metals. In another study, physicochemical analyses of Ink wastewater revealed the presence of Copper (Cu), Nickel (Ni) and Lead (Pb) [11]. The presence of traces of metals particularly heavy metals was believed to hinder seed water absorption causing inadequate mobilization of seed reserve content [11].

In *Zea mays,* root length was significantly reduced in treated contaminated RBB and AP textile dyes water compared with the deionised water (control). However, there was no significant difference in the root length of *Zea mays* among the various concentrations of RBB and AP textile dyes treatments (Tables 2 & 3). In this study, germination was defined as the emergence of a seed of a radicle. Therefore, the non-appearance of root and shoot in *Sorghum bicolor* germinated seed have suggested the inhibition of the radicle to further develop into distinct root and shoot (Tables 4 & 5). Cadmium contamination has prevented carbohydrate hydrolysis and translocation of hydrolyzed sugars, which results in a significant decrease in shoot height and root length [17].

With the clear evidence of indiscriminate discharging of textile dyes wastewater from the local factories around the study area (Fig. 1), this unhealthy act could lead to complex bonds with the natural soil binders to slow down the normal hydration and increase the release of heavy metals in groundwater [18]. Therefore, it would be of interest to profile the different textile dyes contaminated water to the presence of heavy metals, and also study the bimolecular mechanism of seed germination in RBB and AP contaminated textile dyes water.

4. CONCLUSION

Nigeria, particularly Northern Nigeria have many local textile dyes factories operating, indiscriminately discharging wastewater into the environment. In this study, we conclude that Remazol brilliant blue (RBB) and Aniline purple (AP) textile dyes contaminated water at various concentrations have shown a relative inhibitory effect on seed germination percentage and seedling development. This could be due to the presence of toxic organic and inorganic, and traces of heavy metals. Therefore, from the finding of this study, Remazol brilliant blue and Aniline purple textile dyes contaminated water poses a serious public health risk when allow entering into the food chain. However, an attempt should be made by others to evolve environmentally sustainable wastewater treatment technologies based on actual field data with economic viability.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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